Case Report

Hydrogen-Oxygen Nanobubble Therapy as a Complementary Approach in a Female Patient with Duchenne Muscular Dystrophy (DMD): A Case Report

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Posted Date: 11 September 2025

doi: 10.20944/preprints202509.1031.v1

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Hydrogen-Oxygen Nanobubble Therapy as a Complementary Approach in a Female Patient with Duchenne Muscular Dystrophy (DMD): A Case Report

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Abstract

Background: Duchenne Muscular Dystrophy (DMD) is a severe X-linked neuromuscular disorder characterized by progressive muscle weakness due to mutations in the dystrophin gene. Although predominantly affecting males, rare cases have been reported in females. Current standard management with corticosteroids improves function but is limited by significant long-term adverse effects. Therefore, novel complementary therapies with fewer side effects are needed. **Case Presentation:** We report the case of an 18-year-old female with genetically confirmed DMD presenting with progressive lower limb weakness, difficulty standing, waddling gait, and positive Gower's sign. The patient underwent 20 sessions of intravenous hydrogen-oxygen nanobubble (NB-HHO) therapy in combination with physiotherapy and nutritional supplementation. After 10 sessions, she demonstrated improved sit-to-stand time (<20 seconds), negative Gower's sign, and enhanced lower limb muscle strength (from 4/5 to 5/5). Upon completion of 20 sessions, the patient was able to ambulate independently without assistive devices and actively participate in daily and academic activities. **Discussion:** This case highlights the potential role of NB-HHO therapy in reducing oxidative stress and inflammation—two key mechanisms in DMD progression—while supporting mitochondrial function and oxygen delivery. The observed clinical improvements suggest that NB-HHO may serve as an effective complementary therapy to enhance rehabilitation outcomes in DMD. However, its safety and efficacy require validation in larger clinical trials. **Conclusion:** Hydrogen-oxygen nanobubble therapy, in combination with conventional rehabilitation, may represent a promising adjunctive approach for female DMD patients. Further research is warranted to establish its therapeutic potential and long-term benefits.

Keywords: duchenne muscular dystrophy; female; nanobubble therapy; hydrogen-oxygen; rehabilitation

Introduction

Duchenne Muscular Dystrophy (DMD) is a severe and progressive neuromuscular disorder (1,2), characterized by gradual muscle damage and weakness (3). This disease is caused by mutations in the dystrophin gene (which encodes the dystrophin protein), leading to the absence of dystrophin production in muscles (2,4,5). Muscles lacking dystrophin become more vulnerable to damage, resulting in continuous muscle tissue loss and functional decline, and may also trigger cardiomyopathy (2,6). Early symptoms of DMD include difficulty climbing stairs, waddling gait, and frequent falls, typically appearing at 2–3 years of age. Most patients become wheelchair-dependent by the age of 10–12 years and require ventilatory support around the age of 20 years. Even with optimal care, most patients die between the ages of 20 and 40 years due to heart failure and/or respiratory failure (7).

 doi:10.20944/preprints202509.1031.v1

DMD is an X-linked recessive disease, meaning that the majority of affected individuals are male (8). The disease is inherited from mothers who are either asymptomatic or have mild symptoms. If a man with DMD has daughters, they will become carriers of the defective DMD gene and may pass it on to their sons in the next generation. Therefore, this disease often appears in an intergenerational hereditary pattern (1). The global incidence of DMD is estimated at approximately 1 in 3,600 to 5,000 live male births, making it one of the most common hereditary neuromuscular disorders (8).

The DMD gene, which encodes the dystrophin protein, is located on the X chromosome at position Xp21.1. It is one of the largest genes in the human body, spanning about 2.5 megabases (Mb) of DNA and consisting of 79 exons and 78 introns. Its 14-kilobase (kb) mRNA transcript produces a dystrophin protein with a molecular weight of approximately 427 kilodaltons (kDa), comprising 3,685 amino acids (9). Dystrophin functions as a structural bridge between cytoskeletal F-actin and the extracellular matrix, maintaining muscle membrane stability during contraction (7,10). Complete loss of dystrophin function destabilizes the connection between actin filaments within muscle cells and the extracellular framework (3).

Mutations in the DMD gene can disrupt the production of the muscle isoform of dystrophin (Dp427m). These mutations include frameshift mutations (deletions or duplications involving a number of nucleotides not divisible by three) or nonsense mutations (substitution of an amino acid codon with a stop codon). Consequently, premature termination of protein synthesis occurs, resulting in non-functional and unstable dystrophin (7,10). Furthermore, the large size of the DMD gene contributes to a relatively high mutation rate. About one-third of mutations in this gene occur de novo (spontaneous mutations not inherited), while the rest result from germline mosaicism or are inherited from carrier mothers (11).

Diagnosis

Diagnosis of DMD must be performed as early and accurately as possible to ensure appropriate management. The process begins when a child presents with early symptoms such as muscle weakness, difficulty climbing stairs, toe-walking, or a positive Gowers' sign. Prompt referral to a neuromuscular specialist and geneticist can prevent diagnostic delays (12). Experts emphasize that rapid and precise identification of DMD is the key to optimizing patient care (6). Modern diagnostic approaches for DMD include clinical manifestations, laboratory tests, instrumental examinations, molecular-genetic testing, and morphological biopsy analysis (1).

Laboratory tests include biochemical blood assays to measure levels of Creatine Kinase (CK), Alanine Transaminase (ALT), Aspartate Transaminase (AST), and Lactate Dehydrogenase (LDH) (13). In some cases, developmental delay or elevated levels of enzymes such as ALT, AST, LDH, and CK may serve as early indicators of DMD (14). CK is an intracellular enzyme involved in energy metabolism and is found in highest concentrations in cardiac and skeletal muscles. Because it is localized within cells, elevated CK levels in the blood indicate muscle cell damage. Elevated CK is considered an early and definitive marker of DMD, even before clinical symptoms appear. An increase of fivefold or more is regarded as diagnostically significant. In DMD, enzyme levels can rise 10 to 100 times above normal in the early stages of the myodystrophic process, reflecting progressive muscle damage (15).

ALT and AST are concentrated primarily in liver cells (hepatocytes), but they are also found in large amounts in muscle cells. Elevated ALT and AST levels may indicate muscle cell damage (cytolysis). In some cases, increases in ALT and AST may be the only clinical signs, but more often they occur alongside CK elevation. In DMD, these enzymes can rise from the early stages of the disease, with case reports showing ALT levels reaching 477 IU/L and AST up to 497 IU/L (16). LDH is an intracellular enzyme involved in the conversion of lactate to pyruvate and exhibits high activity in skeletal muscle. Elevated LDH in the blood indicates tissue damage and cell destruction, making it an important biomarker in various diseases, including muscular dystrophy (1).

Electroneuromyography (EMG) is an examination method used to assess the electrical activity of muscles and nerves. In DMD, needle EMG is more accurate than stimulation EMG, as it can detect

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muscle weakness characterized by reduced muscle electrical activity and abnormal signals, such as small fibrillations in muscle fibers. These abnormal electrical activities can already be observed in the early stages of DMD, making EMG a valuable tool for detecting and monitoring disease progression. Since DMD results from mutations in the DMD gene, which is responsible for producing dystrophin, genetic testing is essential for diagnosis, treatment planning, and genetic counseling. Mutation detection is typically performed using the Multiplex Ligation-dependent Probe Amplification (MLPA) method, and if results are negative, further testing with Next-Generation Sequencing (NGS) or Sanger Sequencing is conducted (17). Mutations may include deletions (60–68%), duplications (10–11%), or other small mutations (20–30%). Most mutations are found in exons 6–7, 43–46, and 50–53. Confirmation of these mutations also facilitates prenatal diagnosis, enabling families to understand the risk of disease in their offspring (18). Moreover, a proper understanding of the early signs of DMD is crucial to support early detection and optimal care (14).

Current Management of DMD

Despite significant therapeutic advances over the past 30 years, DMD still has no curative treatment. Nevertheless, multidisciplinary and coordinated medical and rehabilitative approaches focusing on DMD symptoms can improve quality of life (QoL) and extend patient survival (3). DMD not only affects cardiac and skeletal muscles but also causes various extra-muscular manifestations and secondary consequences of muscle weakness; therefore, a coordinated, multidisciplinary approach is required (12).

Traditional treatment strategies for DMD have focused on improving quality of life, including the use of glucocorticoids and physical therapy (3). Management of DMD is complex and requires collaboration across multiple disciplines (multidisciplinary approach) (6,19). One key aspect of management is medical therapy aimed at maintaining or improving muscle function. Recent care guidelines agree that corticosteroids are recommended in the medical management of DMD (19).

At present, corticosteroids are considered the gold standard in DMD because they have been proven to slow disease progression and extend patients' functional abilities by more than two years (20). Existing supportive therapies aim to alleviate symptoms and delay disease progression, but they cannot address the underlying cause of DMD or prevent progression to more severe stages, such as loss of ambulation and wheelchair dependence. Patient and parental education regarding the benefits, side effects, and adherence to treatment is a crucial step. Although supportive therapies can help reduce symptoms, they cannot eliminate the primary cause of DMD or prevent further disease progression. The side effects of corticosteroids—such as weight gain, increased fracture risk, behavioral disorders, and hypertension—must be carefully considered in their use. Not all patients can tolerate steroid therapy, leaving them without other treatment options and at risk of poor prognosis (6).

Due to these side effects, which may ultimately worsen the condition of DMD patients, the development of therapies with minimal adverse effects is necessary. Several novel therapeutic approaches have been explored that are expected to address DMD symptoms, including two strategies that are nearing clinical application: stop codon read-through and exon skipping. Stop codon read-through has been developed to facilitate gene translation in patients with nonsense mutations (6), while exon skipping enables the production of shorter but still functional dystrophin. In addition, a new method currently under development involves the administration of hydrogen-oxygen nanobubbles (21).

Utilization of Hydrogen-Oxygen Nanobubbles as a Novel Therapy

Hydrogen-oxygen nanobubbles (HHO) represent an innovative approach as a complementary therapy for DMD. This method involves the administration of nanosized bubbles filled with hydrogen and oxygen gases, which have demonstrated potential therapeutic effects in various medical conditions, including reducing oxidative stress, alleviating hypoxia, supporting cancer

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therapy, and improving vascular health. Preliminary studies have shown promising results, but further research is required to validate their effectiveness on a larger clinical scale. This therapy involves the intravenous infusion of hydrogen-oxygen nanobubble solution into the bloodstream. The nanobubbles are designed to be sufficiently small to pass through capillaries without obstructing blood flow, ensuring targeted delivery and efficient absorption (22).

HHO therapy has the potential to aid in the treatment of DMD, as it can reduce oxidative stress (23) and inflammation, which are the main causes of muscle damage in this disease. Hydrogen possesses strong antioxidant properties capable of neutralizing free radicals (23), while oxygen helps increase oxygen supply to damaged tissues, thereby contributing to the slowing of muscle degeneration. In addition to its antioxidant role, hydrogen in nanobubbles also helps suppress inflammation by reducing levels of pro-inflammatory cytokines such as IL-6, which are often elevated in DMD patients. Oxygen in this therapy also contributes to improved mitochondrial function, enhanced cellular energy production, and increased blood circulation, which may help patients preserve muscle strength for longer. The advantage of HHO therapy is that it can be combined with conventional treatments, potentially enhancing overall effectiveness without causing side effects seen in some pharmacological drugs, while providing a holistic approach that may complement or even augment the efficacy of existing therapies (23).

Case Presentation

The patient, ZRQ, an 18-year-old female, was diagnosed with DMD based on clinical history and examination findings. To manage her condition, the patient underwent high-dose Hydrogen-Oxygen Nanobubble (NB-HHO) therapy. In addition, she was given IFA + Magnesium supplementation. As part of long-term management, she also received medical rehabilitation and physiotherapy, which helped improve muscle strength, mobility, and functional capacity in daily activities.

Pre-therapy complaints included progressive muscle weakness since November 2021, leading to difficulty standing and walking. She also reported pain in both thighs and weakness in both legs, particularly when attempting to stand or walk. Trauma history revealed that she had fallen on her buttocks in November 2021, and a radiograph taken in December 2021 showed a fracture in the knee. The patient had previously undergone physiotherapy at RKZ, but with no significant improvement.

At the initial examination, the patient demonstrated a positive Gower's sign, indicating difficulty rising from a sitting position. Physical examination revealed upper extremity muscle strength of 5/5, while lower extremity strength was 4/4. Reflexes were within normal limits (+2/+2), with no pathological reflexes. Vital signs included blood pressure 112/68 mmHg, heart rate 105 beats per minute, respiratory rate 21 breaths per minute, and oxygen saturation 99%. After 10 sessions of HHO nanobubble therapy (July 27, 2024), the patient began to show significant improvement in mobility. She was able to transition more quickly from sitting cross-legged to standing and walked more smoothly for a distance of 5 meters from a sitting position on a chair. The sit-to-walk test time was less than 20 seconds, indicating motor improvement. In addition, the Gower's sign became negative, suggesting improved muscle strength, and she was assessed as having made very good progress. On physical examination, lower extremity muscle strength improved to 5/5, indicating full recovery of strength. Gait assessment showed greater stability, and she was able to walk without assistive devices.

After completing 20 sessions of therapy (October 18, 2024), the patient experienced significant improvement in functional ability. She could now walk independently without a cane, with faster transitions from sitting to standing and without using her hands. The patient also began actively participating in activities outside of school, including attending a two-week job training program at the Department of Manpower, without difficulty in performing daily activities. Although substantial improvement was noted, the patient still experienced mild hip pain, likely due to muscular adaptation to her new walking pattern. Overall, however, her condition was very fit, and she was more enthusiastic in daily activities. On physical examination, lower extremity strength remained stable at 5/5, reflexes were within normal limits, and the Gower's sign remained negative. Vital signs

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were stable, with blood pressure 112/71 mmHg, heart rate 82 beats per minute, respiratory rate 23 breaths per minute, and oxygen saturation 99%. The final results of the therapy indicated that the patient experienced remarkable progress in mobility, muscle strength, and daily functioning after undergoing 20 sessions of NB-HHO therapy combined with supportive rehabilitation. She is now able to walk independently without assistive devices, transition positions more quickly, and actively participate in academic activities as well as job training.

Discussion

DMD is an X-linked recessive genetic disorder, primarily caused by deletions, duplications, and point mutations in the DMD gene, and generally affects males since they only possess one X chromosome (17). If a mutation occurs in the DMD gene, there is no second X chromosome to compensate, resulting in impaired dystrophin production. Dystrophin is an essential protein that helps maintain muscle stability and function; without it, muscles undergo progressive weakness and degeneration from an early age (24).

However, in rare cases, DMD can also occur in females, as seen in the case of patient ZRQ. This may result from several genetic mechanisms, such as X-inactivation skewing (25), in which the healthy X chromosome is more frequently inactivated compared to the mutated one (26), thereby disrupting dystrophin production. In addition, Turner syndrome (45, X), in which a female has only one X chromosome, can also lead to DMD if that chromosome carries a dystrophin gene mutation (27). Another possibility is a de novo mutation, which occurs spontaneously during fetal development without a family history of DMD (28,29). To confirm a diagnosis of DMD in females, genetic testing is required, along with measurement of creatine kinase (CK) levels, which are generally elevated in DMD patients due to progressive muscle damage. In the case of ZRQ, clinical examination revealed progressive muscle weakness and a positive Gower's sign, which is a hallmark of DMD. Once the diagnosis was established, the patient underwent innovative therapy with hydrogen-oxygen nanobubbles (HHO) to support recovery.

HHO therapy represents a novel approach designed to reduce oxidative stress and inflammation, two key factors in DMD progression. Hydrogen has strong antioxidant properties capable of neutralizing free radicals (30,31). When free radical levels in the body exceed the neutralizing capacity of antioxidants, oxidative stress occurs (32). This oxidative stress can damage macromolecules such as nucleic acids, proteins, and lipids (33), leading to genetic and epigenetic alterations at the molecular level (32), ultimately resulting in cell and tissue damage, accelerating aging, and contributing to the progression of chronic and degenerative diseases (33). Therefore, antioxidants such as hydrogen, delivered in nanobubble form, play a vital role in reducing free radicals (34), particularly for DMD patients, with the potential to minimize muscle damage.

Hydrogen in nanobubbles also helps suppress muscle inflammation by reducing levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) (34), which are often elevated in DMD patients, as IL-6 is closely associated with the pathogenesis of muscular dystrophy. A study by Pelosi et al. (2015) reported that IL-6 expression in adult mdx mice reproduced severe DMD phenotypes observed in humans. Elevated IL-6 levels worsened dystrophic muscle phenotypes by sustaining inflammatory responses and muscle degeneration (35).

Oxygen in this therapy also aids in improving mitochondrial function, as oxygen plays an essential role in the mitochondrial electron transport chain, enabling energy production in the form of adenosine triphosphate (ATP) via oxidative phosphorylation. Adequate oxygen supply ensures optimal mitochondrial function, preventing impaired energy production due to hypoxia. With sufficient oxygen availability, mitochondria also reduce the production of reactive oxygen species (ROS), which contribute to oxidative stress (36,37). Furthermore, oxygen helps enhance tissue oxygenation in muscle (38), thereby supporting cell regeneration and energy metabolism. This therapy is administered intravenously, allowing rapid systemic distribution. Consequently, HHO therapy may significantly increase blood oxygen availability, improve mitochondrial function, and enhance cellular energy production in conditions with metabolic dysfunction, such as DMD.

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Although this therapy is still under investigation, early results in patient ZRQ demonstrated significant improvement. After 20 sessions of HHO therapy, she experienced enhanced muscle strength, improved mobility, and resolution of Gower's sign, reflecting improved muscle stability. She is now able to ambulate without a cane, rise from sitting without hand support, and has returned to academic and vocational activities. These findings suggest that HHO therapy could be an effective adjunctive treatment in DMD management.

The regulation of HHO therapy still requires further clinical trials to confirm its safety, efficacy, and optimal dosing before it can be adopted as a standard treatment for DMD. Compared to corticosteroids—the current mainstay of DMD treatment but associated with numerous long-term side effects, such as increased risk of vertebral and lower limb fractures (39), obesity, cataracts, and growth retardation (40)—HHO therapy offers a safer approach with minimal adverse effects. The success of therapy in patient ZRQ provides preliminary evidence that combining HHO with physiotherapy and other supportive therapies can yield more optimal outcomes for DMD patients. With the growing research on DMD in females and the exploration of innovative therapies such as hydrogen-oxygen nanobubbles, more effective and safer treatment methods are expected to emerge. The case of patient ZRQ exemplifies that appropriate medical management, when combined with innovative therapies, can achieve better outcomes and improve quality of life in DMD patients. Therefore, further research and regulatory development of HHO therapy are needed to enable its widespread use and provide new hope for patients with rare presentations of DMD in females.

Collectively, data from this study indicate that nanobubble therapy is promising and may reduce side effects compared to some traditional pharmacological treatments, offering a safer, more targeted, and potentially more effective therapeutic approach (23). Hydrogen-oxygen nanobubbles present a new and promising pathway for DMD treatment, with the potential to address several fundamental pathological aspects of the disease.

Conclusion

Although HHO nanobubble therapy shows promising potential in the treatment of DMD, its clinical effectiveness has not yet been established. Preliminary studies suggest benefits in reducing oxidative stress and inflammation, but further large-scale clinical trials are required to confirm its safety and efficacy as a complementary therapy for DMD patients. With the advancement of nanotechnology in the form of hydrogen-oxygen nanobubbles as a complementary treatment for DMD, the future of managing this disease is expected to experience significant progress.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Chunkayeva D, Jaxybayeva A. A review of diagnosis of Duchenne and Becker muscular dystrophy. J Clin Med Kazakhstan. 2021;18(5):6–10.
2. Duan D, Goemans N, Takeda S, Mercuri E, Aartsma-Rus A. Weaning strategies and protocols. Non-invasive Vent Weaning Princ Pract [Internet]. 2010;7(13):543–9. Available from: https://doi.org/10.1038/%0As41572-021-00248-3
3. Limback KA, Jacobus WD, Wiggins-McDaniel A, Newman R, White RA. A Comprehensive Review of Duchenne Muscular Dystrophy: Genetics, Clinical Presentation, Diagnosis, and Treatment. Biotechnol J Int. 2022;26(6):1–31.
4. Bez Batti Angulski A, Hosny N, Cohen H, Martin AA, Hahn D, Bauer J, et al. Duchenne muscular dystrophy: disease mechanism and therapeutic strategies. Front Physiol. 2023;14(June):1–31.
5. Roy S, Shaw R, Samaddar S, Samanta S, Maity R, Chatterjee P. Duchenne Muscular Dystrophy: A Review on Systemic Paradigm Approaching Diagnosis to Therapy. J Genet Syndr Gene Ther [Internet].

 doi:10.20944/preprints202509.1031.v1

2024;15(1000413):413. Available from: https://www.researchgate.net/publication/378804746

6. Jumah M Al, Muhaizea M Al, Rumayyan A Al, Saman A Al, Shehri A Al, Cupler E, et al. Current Management of Duchenne Muscular Dystrophy in the Middle East: Expert Report. Neurodegener Dis Manag. 2019;9(3):123–33.
7. Mercuri E, Bönnemann CG, Muntoni F. Muscular dystrophies. Lancet [Internet]. 2019;394(10213):2025–38. Available from: http://dx.doi.org/10.1016/S0140-6736(19)32910-1
8. Venugopal; V, Pavlakis. S. Duchenne Muscular Dystrophy [Internet]. StatPearls Publishing. 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482346/?
9. Shastry A, Aravind S, Sunil M, Ramesh K, Ashley B, Nithyanandan T, et al. Matrilineal analysis of mutations in the DMD gene in a multigenerational South Indian cohort using DMD gene panel sequencing. Mol Genet Genomic Med. 2021;9(5):1–12.
10. Aartsma-Rus A, Van Deutekom JCT, Fokkema IF, Van Ommen GJB, Den Dunnen JT. Entries in the Leiden Duchenne muscular dystrophy mutation database: An overview of mutation types and paradoxical cases that confirm the reading-frame rule. Muscle and Nerve. 2006;34(2):135–44.
11. Nallamilli BRR, Chaubey A, Valencia CA, Stansberry L, Behlmann AM, Ma Z, et al. A single NGS-based assay covering the entire genomic sequence of the DMD gene facilitates diagnostic and newborn screening confirmatory testing. Hum Mutat. 2021;42(5):626–38.
12. Birnkrant DJ, Bushby K, Bann CM, Alman BA, Apkon SD, Blackwell A, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. Lancet Neurol. 2018;17(4):347–361.
13. Gaynetdinova DD, Novoselova, A A. Current diagnosis and treatment of Duchenne muscular dystrophy. Kazan Med J. 2020;101(4):530–537.
14. Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol [Internet]. 2018;17(3):251–67. Available from: http://dx.doi.org/10.1016/S1474-4422(18)30024-3
15. Aghayeva S, Mamedbeyli A, Rasulov E. Creatinkinase activities polymorphism study in three families with Duchenne muscular dystrophy from Azerbaijan republic. Paediatr child Surg journal. 2018;4(94):73–6.
16. Wright MA, Yang ML, Parsons JA, Westfall JM, Yee AS. Consider muscle disease in children with elevated transaminase. J Am Board Fam Med. 2012;25(4):536–40.
17. Aravind S, Ashley B, Mannan A, Ganapathy A, Ramesh K, Ramachandran A, et al. Targeted sequencing of the DMD locus: A comprehensive diagnostic tool for all mutations. Indian J Med Res. 2019;150:282–9.
18. Xu Y, Li Y, Song T, Guo F, Zheng J, Xu H, et al. A retrospective analysis of 237 Chinese families with Duchenne muscular dystrophy history and strategies of prenatal diagnosis. J Clin Lab Anal. 2018;32(7):1–8.
19. Rivera SR, Jhamb SK, Abdel-Hamid HZ, Acsadi G, Brandsema J, Ciafaloni E, et al. Medical management of muscle weakness in Duchenne muscular dystrophy. PLoS One [Internet]. 2020;15(10 October):1–10. Available from: http://dx.doi.org/10.1371/journal.pone.0240687
20. Scully MA, Cwik VA, Marshall BC, Ciafaloni E, Wolff JM, Getchius TS, et al. Can outcomes in duchenne muscular dystrophy be improved by public reporting of data? Neurology. 2013;81(20):1802.
21. Mendell JR, Lloyd-Puryear M. Report Of MDA Muscle Disease Symposium On Newborn Screening For Duchenne Muscular Dystrophy. Muscle and Nerve. 2013;48(1):21–6.
22. Hernowo AT, Pardosi JC, Subagjo S, Dewi ATK, Sumitro SB. Partial Reversal of Coronary Stenosis with Intravenous Hydrogen and Oxygen Nanobubble Administration : A Case Report Partial Reversal of

 doi:10.20944/preprints202509.1031.v1

Coronary Stenosis with. 2024;

23. Hernowo AT, Widyarti S, Aristyani S. Evaluating the safety and therapeutic efficacy of intravenous hydrogen nanobubble infusions in a hypercholesterolemic rat model. Berk Penelit Hayati. 2022;30(1):40–7.
24. Falzarano MS, Scotton C, Passarelli C, Ferlini A. Duchenne muscular dystrophy: From diagnosis to therapy. Molecules. 2015;20(10):18168–84.
25. Sun MX, Jing M, Hua Y, Wang JB, Wang SQ, Chen LL, et al. A female patient carrying a novel DMD mutation with non-random X-chromosome inactivation from a DMD family. BMC Med Genomics. 2024;17(1):1–7.
26. Viggiano E, Ergoli M, Picillo E, Politano L. Determining the role of skewed X-chromosome inactivation in developing muscle symptoms in carriers of Duchenne muscular dystrophy. Hum Genet. 2016;135(7):685–98.
27. Satre V, Monnier N, Devillard F, Amblard F, Lunardi J. Prenatal diagnosis of DMD in a female foetus affected by Turner syndrome. Prenat Diagn. 2004;24(11):913–7.
28. Strmecki L, Hudler P, Benedik-Dolničar M, Komel R. De novo mutation in DMD gene in a patient with combined hemophilia A and Duchenne muscular dystrophy. Int J Hematol. 2014;99(2):184–7.
29. Yu H, Chen YC, Liu GL, Wu ZY. A de novo mutation in dystrophin causing muscular dystrophy in a female patient. Chin Med J (Engl). 2017;130(19):2273–8.
30. Huang CS, Kawamura T, Toyoda Y, Nakao A. Recent advances in hydrogen research as a therapeutic medical gas. Free Radic Res. 2010;44(9):971–82.
31. Yang W, Wang Q. AMS-H-03 Hydrogen-oxygen therapy improves postoperative pulmonary functions and accelerates recovery through attenuating inflammatory reactions and oxidative stress in patients undergoing lung surgery Take-home Message. Res Sq. 2022;
32. Afzal S, Abdul Manap AS, Attiq A, Albokhadaim I, Kandeel M, Alhojaily SM. From imbalance to impairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. Front Pharmacol. 2023;14(October):1–22.
33. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. Oxygen. 2022;2(2):48–78.
34. Xiao L, Miwa N. Hydrogen nano-bubble water suppresses ros generation, adipogenesis, and interleukin-6 secretion in hydrogen-peroxide-or pma-stimulated adipocytes and three-dimensional subcutaneous adipose equivalents. Cells. 2021;10(3):1–14.
35. Pelosi L, Berardinelli MG, Forcina L, Spelta E, Rizzuto E, Nicoletti C, et al. Increased levels of interleukin-6 exacerbate the dystrophic phenotype in mdx mice. Hum Mol Genet. 2015;24(21):6041–53.
36. Nolfi-Donegan D, Braganza A, Shiva S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. Redox Biol [Internet]. 2020;37:101674. Available from: https://doi.org/10.1016/j.redox.2020.101674
37. ZHAO R, JIANG S, ZHANG L, YU Z. Mitochondrial electron transport chain, ROS generation and uncoupling. Int J Mol Med. 2019;44:3–15.
38. Porter MS, Reed K, Jones B. The use of acute oxygen supplementation upon muscle tissue saturation during repeat sprint cycling. J Hum Sport Exerc. 2020;17(1):1–12.

 doi:10.20944/preprints202509.1031.v1

39. King WM, Ruttencutter R, Nagaraja HN, Matkovic V, Landoll J, Hoyle C, et al. Orthopedic outcomes of long-term daily corticosteroid treatment in Duchenne muscular dystrophy. Neurology. 2007;68(19):1607–13.
40. Schara U, Mortier J, Mortier W. Long-Term Steroid Therapy in Duchenne Muscular Dystrophy - Positive Results versus Side Effects. Vol. 2, Journal of Clinical Neuromuscular Disease. 2001. p. 179–83.